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Shorter research note:

Drilling discharges reduce sediment reworking of two benthic species

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Abstract

Effects of water-based drill cuttings on sediment reworking activity were studied on two important benthic bioturbators (the bivalve *Abra segmentum* and the brittle star *Amphiura filiformis*) using thin aquaria, fluorescent-dyed sediment particles (luminophores), time lapse photography and image analysis. In the present context, sediment reworking activity was measured as maximum mixing depth and total amount of luminophores transported below the sediment-water interface. There was a significant reduction in the amount of downward transported luminophores in drill cuttings treatments compared to controls with added natural sediments for both species, which also was true regarding maximum mixing depth for *A. segmentum*. Further, *A. filiformis* showed a clearly delayed burrowing of luminophores in the drill cuttings treatment compared to control. To conclude, the study showed that water-based drill cuttings have the potential to reduce sediment reworking. Further, it is evidenced that water-based drill cuttings not only cause burial effects.

Key words: *Abra segmentum*, *Amphiura filiformis*, bioturbation, drill cuttings, luminophores

Offshore drilling activities discharge large amounts of drill cuttings which accumulate on the seabed where they may cause environmental harm on benthic organisms. Drill cuttings are mainly particles of crushed rock, but also contain remnants of drilling muds which consist of a base fluid, weighting agents, bentonite clay as well as various additives (Neff et al., 1987). The base fluid is water, diesel, mineral oil or a synthetic compound. Most drilling in the North Sea, the Gulf of Mexico, and other production areas is currently performed with water-based muds (WBM), due to the large environmental impacts caused by oil-based or synthetic muds. In general, cuttings with associated water-based muds are assumed to have low toxicity, and the main effect on

benthic fauna is considered to be burial, although this assumption has been questioned (Trannum et al., 2010).

Numerous field and mesocosm studies have been performed to assess effects of drill cuttings on benthic community structure (Kingston, 1987; Gray et al., 1990; Olsgard and Gray, 1995; Daan and Mulder, 1996; Schaanning et al., 2008; Trannum et al., 2010), but there is a lack of information when it comes to functioning. In the understanding of benthic ecosystem functioning, analysis and quantification of sediment reworking (bioturbation) is crucial. Bioturbation can be defined as the result of burrowing, feeding, irrigating, respiring, and defecating activities of benthic species (Rhoads, 1974), and is a key mediator of several important geochemical processes in marine systems (Queiros et al., 2013). Bioturbation (including burrow ventilation) is a significant transport process of solids and solutes including organic matter and nutrients in the upper sediment layer (Aller, 1982), and also provides a mechanism for incorporating pollutants into the substrate or remobilise those that are buried (Bradshaw et al., 2006).

The aim of the present study was to investigate how drilling discharges influence sediment reworking of two benthic species; the brittle star *Amphiura filiformis* (O.F. Müller, 1776) and the bivalve *Abra segmentum* (Récluz, 1843). *A. filiformis* is a common species in northeast Atlantic and Mediterranean soft bottom communities, at depths down to 200 m (Rosenberg and Lundberg, 2004). It is typically abundant in densities of >100 individuals m^{-2} (including Norwegian oil fields), but may also reach >1000 individuals m^{-2} (Josefson, 1998). The disc of *A. filiformis* is usually buried 4-8 cm beneath the sediment surface, while its arms are stretched through the sediment to feed. The species is able to switch between surface deposit feeding and suspension feeding, depending on the hydrodynamic conditions and organic matter availability at the sediment surface (Buchanan, 1964; Solan and Kennedy, 2002). The species is characterized as a key species (O'Connor et al., 1983). This also applies when it comes to its role as an ecosystem engineer since it masks the bioturbatory signatures of other species (O'Reilly et al., 2006; Solan and Kennedy, 2002). The euryhaline bivalve *Abra segmentum* is widespread in e.g. the Mediterranean and Caspian sea, inhabiting sandy mud, where it

can be found in densities of >1000 individuals m^{-2} (Guelorget and Mayere, 1981). It is a surface deposit feeder bivalve which lives buried in the upper part of the sediment and uses its siphon for feeding (Guelorget and Mayere, 1981). As it is an efficient sediment reworker, it contributes to a large proportion of sediment reworking rate of the entire community (Maire et al., 2006). Both species were sampled in the NW Mediterranean; *Amphiura filiformis* with a dredge at ~ 30 m depth and *Abra segmentum* by hand-collection in a shallow bay. They were then acclimated to a temperature of 15-17 °C and a salinity of 37.0-37.6 ‰ within approximately 12 hours.

Sediment reworking activity (bioturbation) was examined non-invasively using fluorescent-dyed sediment particles (luminophores) and profile imaging. Twelve thin aquaria (8 mm Plexiglas, inner dimension of 1.5 x 17 x 30 cm, water supply 50 ml min^{-1} , 15-18 °C/36.6-37.6 ‰), filled with 15 cm of defaunated sediment, were used in each experiment. Two specimens of *Amphiura filiformis* (~ 800 ind. m^{-2}) and three specimens of *Abra segmentum* (~ 1200 ind. m^{-2}) were used pr. aquaria. One day after addition of test organisms, either drill cuttings or natural sediment were added to a layer thickness of 2.5 mm, i.e. $n=6$ (Table 1). The drill cuttings were water-based with ilmenite ($FeTiO_3$) as weighting material, and originated from a Norwegian drilling operation. The natural test sediment added was the same as that used to fill the aquaria. Four days after addition of test materials, 3 g luminophores were added (green/yellow colour, density 1936 $kg\ m^{-3}$ in water, median size 39 μm). At day two (48h), the organisms were fed with phytodetritus (*Thalassiosira weissflogii*).

Images were captured using a digital Canon EOS 20D Camera under UV light (365 nm) 0, 3, 6, 12, 24 and 48 h after addition of luminophores, and repeated after addition of food (Table 1). After food addition, one set of images was taken after 96 h. One aquarium side was used for the further processing of images (same side for all aquaria, selected *a priori*). Thus, each data series is composed of twelve images. An image analysis of the photos was performed with the program CVABimage software (Duchêne et al., 2000; Duchêne and Nozais, 1994). Images were saved in jpg format, with a size of 3504 x 2336 pixels (one pixel = 0.049 mm) and then transformed to an AVI film. At each image the

sediment-water interface was manually drawn. After this operation a new AVI film was produced based on the “flattened” interface, from which the luminophores in each pixel row (i.e. sediment depth) were summed and the vertical luminophore profiles extracted.

In the present context, two measures were used to estimate mixing activity; maximum luminophore penetration depth (MPD) and sum of all luminophores transported below the sediment-water interface (Σ LUM). The maximum penetration depth is the maximum depth where luminophores were recorded at the aquaria-sediment interface, and corresponds with the maximum depth where mixing activity has been observed. This measure is commonly included in assessment of bioturbation (Norling et al., 2007; Queiros et al., 2015). The sum of all luminophores transported below the sediment-water interface is used as a measure to reflect the availability to transport particles down from the sediment surface. The commonly applied bioturbation coefficient (D_b) is not included in the present work due to lack of fit with bioturbation models for *Amphiura filiformis*. Nevertheless, the selected parameters represent actual measures, independent of model fitting. To test for differences between the two treatments in MPD and Σ LUM, ANOVA was performed (JMP version 6).

Both test species showed a reduced sediment reworking activity in the drill cuttings compared to the natural sediment treatments (Figure 1). For *Abra segmentum* both measures were significantly different between the treatments ($p < 0.001^{***}$ for Σ LUM/MPD). There was a large standard deviation, but nevertheless the pattern was highly consistent and very clear from start.

For *Amphiura filiformis*, Σ LUM was significantly different between the treatments ($p = 0.003^{**}$), while the difference was not significant for MPD. However, Σ LUM is considered a more robust measure for bioturbation than MPD as the MPD is much more influenced by single particles. Interestingly, despite no significant differences between the treatments being found for MPD, the mixing pattern differed, with a clearly delayed downward transportation of luminophores in the drill cuttings compared to the natural sediment treatment. It should also be noted that the standard deviation of MPD was very

low, which means that this pattern was highly consistent in all aquaria. The luminophores were transported to the deepest layer after short time, especially in the natural sediment treatment, and then the rate of increase was subsequently reduced. This reflects the feeding behavior of *A. filiformis*, where surface particles are being transported quickly to the disc through the burrow. No further deeper mixing then takes place.

Effects of drilling discharges on sediment reworking activity has only been sparingly studied previously, and thus there is a lack of scientific literature. In a bioturbation experiment on the community level conducted in a master thesis, Olsson (2006) found significantly shallower mixing in cores from the Barents Sea treated with drill cuttings compared to sediment controls. No significant treatment effects were observed in cores from the Oslofjord, possibly due to a more tolerant fauna caused by more background pollution in this area. Further, a report documents that drill cuttings reduced the burrowing depth of the opportunistic annelid *Capitella capitata* (Woodham et al., 2001). Strømgren (1993) investigated the effect of hydrocarbons and drilling fluids on the fecal pellet production of the deposit feeder *Abra alba*. They found a decrease in fecal pellet production for oil-based mud, while water-based drill cuttings gave a variable, but not significantly different, response from controls. The reduction in fecal pellets production was interpreted as a reduced feeding activity which again was suggested to slow down the bioturbation of the sediments, in accordance with the findings in the present experiment.

Amphiura filiformis is generally sensitive to contamination (Rygg, 1985). In an experiment conducted on the community structure level with similar drill cuttings as in the present experiment, the abundance of *Amphiura filiformis* was reduced in boxes with water-based drill cuttings compared to boxes without addition or with added natural sediment (Trannum et al., 2010). In oilfields drilled with the more environmentally harmful oil-based mud, Olsgard and Gray (1995) found that the density of *A. filiformis* was severely depressed close to the platforms. In contrast to these previous studies, the present study has documented sub-lethal responses of cuttings deposition on this species,

which shows that there might be environmental effects on a larger area than that deduced from abundance data.

Due to the lack of research on this topic, the particular reasons for the reduced mixing in drill cuttings treatments are not fully understood and also outside the scope of this note to discuss in detail. It seems reasonable that there might be a toxic response, limiting energy devoted to feeding and movement. Despite the underlying mechanisms, these responses reduce the capacity of the organism to mix the upper sediment. Both test species contribute to a large proportion of sediment reworking rate of the entire community, which means that the overall bioturbation may be impaired. Such diminished sediment reworking may reduce transport of drill cuttings downwards in the sediment, i.e. a larger fraction will remain on the surface where they can remain as chronic stressors or possibly contribute to a spreading of contaminants to other ecosystem components. Further, the transport of oxygen and nutrients downwards in the sediment may be slowed down, which in concert with degradable compounds in the drill cuttings (Neff, 2005; Trannum et al., 2010) may enhance sediment hypoxia and constrain the vertical distribution of benthic organisms. Another major conclusion of the experiment is that water-based drill cuttings not only cause burial effects, evidenced by different responses in drill cuttings vs. natural sediment additions.

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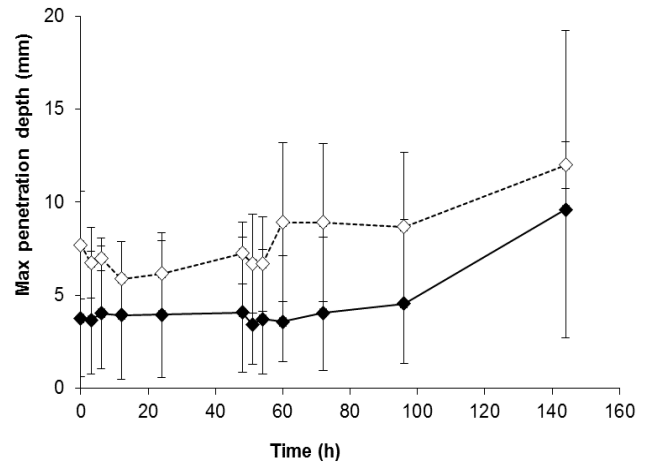
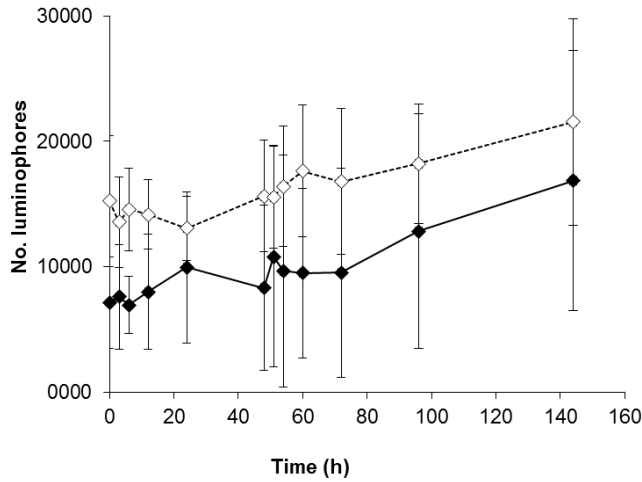
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Table 1. Time-schedule of the thin aquaria experiment.

Day	Time (h)	Activity
-4		Addition of sediment
-2		Addition of test organisms
-1		Addition of cuttings/clean sediment
0	-1	Addition of luminophores
	0	Photo
	3	Photo
	6	Photo
	12	Photo
1	24	Photo
2	48	Photo, addition of food
	51	Photo
	54	Photo
	60	Photo
	72	Photo
3	72	Photo
4	96	Photo
6	144	Photo

a) *Abra segmentum*



b) *Amphiura filiformis*

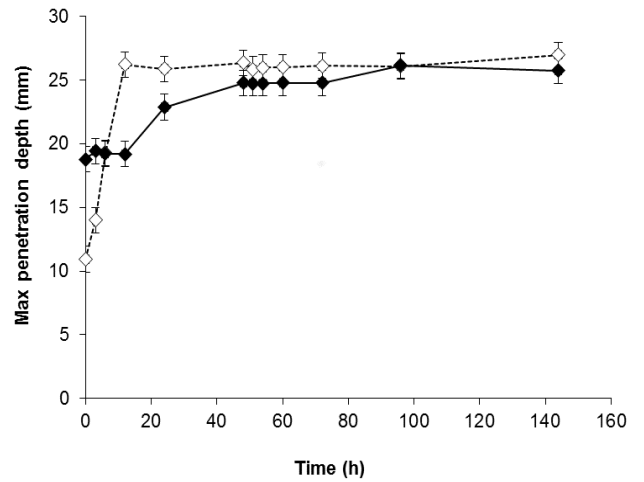
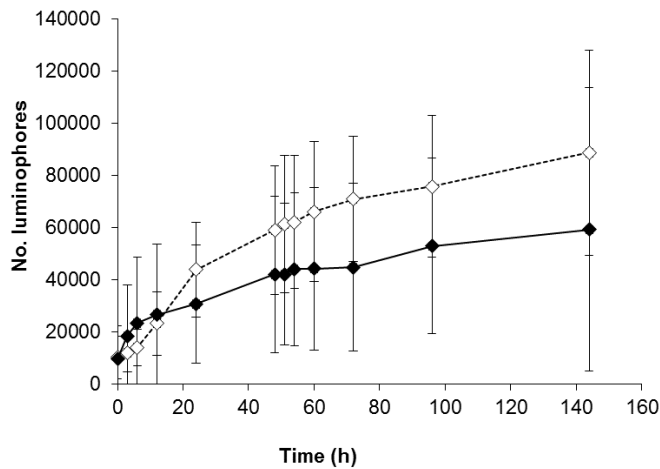


Figure 1. Average total number of luminophores (Σ LUM) transported below the sediment-water interface (\pm SD) and average maximum luminophore penetration depth (MPD) (\pm SD) for *Abra segmentum* (a) and *Amphiura filiformis* (b). Food was added after 48 h. Open bars (\diamond) denote natural test sediment and filled bars (\blacklozenge) denote drill cuttings. n=6.